REMARKS

Claims 4 and 27-33 are currently pending. Claims 4, 30, and 32-33 are amended herein to clarify the claimed subject matter. New claims 34-36 are presented herein. Accordingly, instant claims 4 and 27-36 are under consideration.

Any amendment, however, is not to be construed as abandonment of any subject matter of the originally filed application. Accordingly, it is to be understood that Applicant reserves the right to reintroduce subject matter deleted from the application by the foregoing amendments and to file one or more divisional, continuation, and/or continuation in part applications directed to such subject matter.

For the sake of clarity, support for amendments to the claims is identified using the paragraph numbering set forth in U.S. Application Publication No. 2006-0035233, which corresponds to the present specification.

Support for amendment to the claims is found throughout the specification and in the original claims. More specifically, support for amendment to claim 4 is found, for example, in original claim 4 and in Figure 2, and paragraphs [0062] and [0071], wherein support for amendment to steps (b) and (d) is found; and in paragraph [0069], wherein support for amendment to step (e) is found. Support for amendment to claims 27, 30, and 33 is found, for example, in original claim 4. Support for amendment to claim 32 is found, for example, in previously presented claim 31. No issue of new matter is introduced by these amendments.

Support for new claims 34-36 is found throughout the specification and in the original claims. More specifically, support for new claim 34 is found, for example, in original claim 4 and in paragraph [0069]. Support for new claim 35 is found, for example, in original claim 4 and in Figure 2, and paragraphs [0062], [0071], [0069], [0059], and [0104]. Support for new claim 36 is found, for example, in original claim 4 and in Figure 2, and paragraphs [0062], [0071], [0069], [0059], [0072], and [0102]. No issue of new matter is introduced by these amendments.

Claim Objections

Claim 4 is objected to because the method results in the sequencing of several different nucleic acids, rather than a single nucleic acid. Claim 4 is amended herein to recite "a method for sequencing nucleic acids' in the preamble and to recite "removing the complementary copy of the template sequences" in step (c). It is, therefore, believed that the objection to this claim is obviated.

Rejections under 35 U.S.C. § 112

Claims 30 and 32-33 are rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness. Claims 30 and 32-33 are amended herein to delete the allegedly indefinite subject matter. In view of the amendments to the claims, the rejection, as it applied to claims 4 and 27-32 is obviated.

In view of the amendments to the claims, Applicant respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 USC § 112, second paragraph.

Rejections under 35 USC § 103

Claims 4, 27-28, 30-31, and 33 are rejected under § 103(a) as allegedly unpatentable over Balasubramanisn [*sic*], which should read Balasubramanian et al. (WO 01/57248; published 9/2001) as evidenced by Cheeseman [United States Patent Number (USPN) 5,302,509; issued 1994] in view of Soper et al. (USPN 5,846,727) and Parker et al. (USPN 5,565,323). In view of the clarifying amendments to the claims and arguments presented herein, this rejection is respectfully traversed.

Claim 4 is amended herein to clarify that the claimed method is directed to sequencing a nucleic acid, the method comprising: (a) forming an array of immobilised single-stranded template nucleic acid molecules wherein the density of immobilised single-stranded template nucleic acid molecules is 10^6-10^9 different template sequences per cm^2; (b)determining the sequences of the immobilised single-stranded template nucleic acid molecules by synthesising a first complementary copy of each of the template sequences, wherein said synthesising involves

repeated cycles of incorporating a single nucleotide into the first complementary copy and detecting incorporation of the single nucleotide, thereby performing a first round of sequencing to generate a sequence of the first complementary copy; (c) removing the complementary copy of each of the template sequences from the array; (d) performing a second round of sequencing of each of the immobilised single-stranded template nucleic acid molecules by synthesising a second complementary copy of each of the template sequences, wherein said synthesizing involves repeated cycles of incorporating a single nucleotide into the second complementary copy and detecting incorporation of the single nucleotide to generate a sequence of the second complementary copy; and (e) comparing the sequence of the first complementary copy to the sequence of the second complementary copy for each of the immobilized single-stranded template nucleic acid molecules to confirm sequencing data for each of the immobilized single-stranded template nucleic acid molecules.

The Examiner acknowledges that Balasubramanian et al. do not teach a method further comprising removing the complementary copy of the template sequence and performing a second round of sequencing. The Examiner relies on Soper et al. for teaching a sequencing method wherein after primer extension, the extension products are removed from the immobilized templates by denaturing with mild aqueous alkali. The Office Action refers to column 8, lines 54-61 of Soper et al. for teaching that after extension products have been removed the biotinylated template is ready for "resequencing" if desired. Commentary pertaining to resequencing is only found in the paragraph of Soper et al. comprising lines 54-61 of column 8, wherein it is noteworthy that Soper et al. fail to provide any motivation as to what circumstances would render resequencing desirable. Absent such instructive guidance, it is not apparent what conditions would motivate an ordinarily skilled practitioner to resequence a biotinylated template. Resequencing is, therefore, offered as an optional step of the Soper et al. method with no further explanation.

The Examiner, furthermore, recognizes that the combined teachings of Balasubramanian et al. and Soper et al. do not teach comparing the first and second rounds of sequencing to confirm sequencing data. The Parker et al. reference is, however, relied upon for teaching a

method wherein multiple sequences are obtained and then the sequences are aligned and compared with published sequences. The Examiner points to column 15, lines 50-54 as describing detection of mutations, which were then confirmed by resequencing the variant regions. As addressed in greater detail below, the Parker et al. reference fails to remedy the defects of the combined teachings of Balasubramanian et al., Cheeseman, and Soper et al.

It is noteworthy, moreover, that the Soper et al. reference is directed to a system and method that bear no resemblance to the "system" and method of Balasubramanian et al., which are based on an array of immobilized nucleic acid templates. As detailed, for example, at column 4, line 59 through to column 5, line 20, the system and method of Soper et al. are described as follows:

> *"A novel system has been discovered for the rapid and cost-effective sequencing of DNA. There are three principal components of the novel system: (1) a microreactor, which prepares DNA sequencing "ladders" using solid-phase techniques, preferably in capillary tubes whose volumes are on the order of 10-1000 nanoliters, preferably not more than about 200 nanoliters; (2) a microfabricated electrophoresis capillary separation unit, which separates the components of the sequencing ladders by size; and (3) a fluorescence detector with single-mode optical fibers interfaced directly to the electrophoresis capillary, for detecting and identifying the bases separated in the capillary.*
>
> *The invention is suitable for a highly multiplexed, automated DNA sequencing device. Typical steps in sequencing are as follows: (1) PCR amplification of a DNA template in microtiter dishes using labelled primers, e.g., primers labelled with biotin; (2) immobilizing the labelled PCR products on the walls of one or more capillary tubes having volumes preferably on the order of 10-200 nanoliters; (3) preparing nanoliter quantities of labelled Sanger extension products of the amplified DNA; (4) purifying the oligonucleotide sequencing ladders; (5) high speed electrophoretic separation of the sequencing ladders; and (6) near-infrared, laser-induced fluorescence detection of the*

oligonucleotides. Base-calling is preferably performed in a single lane format with a single fluorophore, in which the bases are distinguished by different fluorescence lifetimes of dyes that otherwise have similar absorption and fluorescence emission spectra at the wavelengths used."

In accordance with the system and method of Soper et al., a plurality of labeled PCR products are produced by PCR amplification of DNA templates, which are then immobilized in a microreactor (typically, a capillary tube) wherein labeled Sanger extension products of the amplified DNA are generated. The labeled Sanger extension products (sequencing ladders) are then separated based on size by the microfabricated electrophoresis capillary separation unit and resolved by gel-based electrophoresis, after which laser-induced fluorescence is used to detect labeled nucleotides incorporated into each sequencing ladder.

The method of Soper et al., therefore, differs substantially from that of Balasubramanian et al. and Cheeseman. At the outset, there is no teaching or suggestion in Soper et al. pertaining to an array of polynucleotide molecules immobilized on a solid support, nor is there any appreciation of the potential for sequencing the immobilized polynucleotide molecules on the array. In contrast, the method of Soper et al. requires multiple formats: a microreactor for synthesis of sequencing ladders, an electrophoresis capillary separation unit to size select sequencing ladders, and ultimately a gel-based electrophoretic unit to achieve base calling of the sequencing ladders.

Based on the disparate essential system components of Balasubramanian et al. and Cheeseman and those of Soper et al. and the consequent absence of <u>any</u> common method steps, Applicant asserts that an ordinarily skilled practitioner would have had no reason to combine the method of Balasubramanian et al. and Cheeseman with the method of Soper et al. to arrive at the present method. The array based methods of Balasubramanian et al. and Cheeseman simply lack a sufficient number of elements and method steps in common with the method of Soper et al. to merit such a combination. Moreover, the Office Action has failed to provide a rationale to support the contention that it would allegedly have been obvious for an ordinarily skilled

practitioner to combine the incongruent methods of Balasubramanian et al. and Soper et al. to arrive at the present invention. More particularly, there is no rationale provided in the Office Action to explain why an ordinarily skilled artisan would modify the method of Balasubramanian et al. to add an optional and ill-defined resequencing step of Soper et al. The Examiner merely offers that the claimed method steps were known in the prior art and one skilled in the art could have arrived at the claimed invention by combining the steps that were taught in the prior art. In this regard, the Examiner is respectfully reminded that while KSR "counsels against applying the [TSM test] as a rigid and mandatory formula... it remains necessary to show 'some articulated reasoning with some rational underpinning to support the legal conclusion on obviousness." *Aventis Pharma v. Lupin* (citing *KSR v Teleflex*). *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007), moreover, requires that an Examiner provide "some articulated reasoning with some rationale underpinning to support the legal conclusion of obviousness." 127 S.Ct. at 1741. An Examiner must "identify a reason that would have prompted a person of ordinary skill in the relevant field to **combine the elements in the way the claimed new invention does**," Id. In light of the above, the Office Action has, therefore, failed to establish a *prima facie* case of obviousness.

An ordinarily skilled practitioner would, furthermore, appreciate that a gel-based sequencing method such as that described by Soper et al. cannot be used to determine "... the sequences of the immobilised single-stranded template nucleic acid molecules by synthesising a first complementary copy of each of the template sequences, wherein said synthesising involves repeated cycles of incorporating a single nucleotide into the first complementary copy and detecting incorporation of the single nucleotide, ..." as recited in the instant claims. Soper et al.'s gel-based sequencing methods are directed to the generation of sequencing ladders that are resolved via electrophoretic means to detect labeled incorporated nucleotides after all incorporation steps are completed. Ongoing incorporation of single nucleotides and detection thereof is not possible in a gel-based format such as that described in Soper et al. Indeed, long sequencing reads are desirable in such applications and Soper et al. teach that typical sequencing ladders are 400-500 bases. See, for example, the Abstract of Soper et al. Moreover, in

approaches utilizing Sanger type sequencing methods, short sequencing reads are problematic. Long sequencing ladders generated by multiple incorporation events are necessary to achieve the clear objective of long sequencing reads as taught by Soper et al. This critical feature of the Soper et al. method illustrates yet another distinction of the method described therein and that of Balasubramanian et al. or Cheeseman. This limitation would have further distanced these distinct methods in the mind of an ordinarily skilled artisan had such an individual conjured any reason to consider these references in combination. It is notable, furthermore, that the above defects of Soper et al. are not remedied by the combined teachings of Balasubramanian et al., Cheeseman, and Parker et al.

Moreover, as alluded to herein above and recited in the claims, the instant method calls for comparing the sequences of the first and second complementary copies of each of the templates immobilized on the array to confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules. In contrast, the method of Soper et al. is performed such that sequencing ladders are generated from any one of a plurality of labeled PCR products immobilized in a microreactor and there is no way to determine which sequencing ladder is an extension product of any particular immobilized labeled PCR product. Should the optional resequencing step be performed in the context of the Soper et al. method, there would be no way of knowing which of the sequencing ladders (i.e., extension products) generated in the resequencing step corresponds to a particular immobilized labeled PCR product. Therefore, there would be no mechanism for determining which sequencing ladder generated in the initial extension reaction correlates with a sequencing ladder generated in the second (resequencing) extension reaction. Thus, resequencing in the context of Soper et al. is only applicable to a second cycle of sequencing a population en masse, but is not capable of yielding any information that can be ascribed to a single particular template in the population. In light of the above, Applicant asserts that the optional resequencing step of Soper et al. could not be used in a way to "confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules" as recited in step (e) of claim 4 because it is not possible to differentiate which sequence ladders derive from which immobilized labeled PCR product. This shortcoming of the

Soper et al. method underscores yet another significant distinction between the method described therein and that of the present invention. That being the case, the alleged motivation to combine Balasubramanian et al., Cheeseman, and Soper et al. to arrive at the claimed method is further undermined by a lack of substantiating rationale. It is also apparent, moreover, that the above deficiencies of Soper et al. are not remedied by the combined teachings of Balasubramanian et al., Cheeseman, and Parker et al.

Turning more particularly to the teachings of Parker et al., the Examiner relies on the Parker et al. reference for teaching a method wherein multiple sequences are obtained and then the sequences are aligned and compared with published sequences. The Parker et al. reference, like Soper et al., is directed to gel-based sequencing methods. See, for example, column 15, lines 46-49. As described herein above, a sequencing ladder analyzed on a sequencing gel cannot be positively ascribed to a specific individual template within the plurality of templates extended to produce sequencing ladders (i.e., extension products) in such protocols. The sequencing ladders of Parker et al. may be generated from any one of a plurality of double-stranded plasmid DNA molecules during the sequencing reaction (see, for example, column 14, line 49 through to column 15, line 2) and there is no way to determine which sequencing ladder is an extension product of any particular double-stranded plasmid DNA molecule. By extension, a resequencing step performed in the context of the Parker et al. method would generate sequencing ladders that could not be positively ascribed to any particular double-stranded plasmid DNA molecule in the pool of double-stranded plasmid DNA molecules present in the sequencing reaction. Sequencing and resequencing in the context of Parker et al. are, therefore, only relevant to sequencing a total population and do not produce any sequence information that can be ascribed to a single particular template in the population of templates. This stands in contrast to the present invention, wherein a first and second round of sequencing can be performed on the same immobilized single-stranded template nucleic acid molecule, thereby making it possible to compare the first and second rounds of sequencing to confirm sequencing data for each of the immobilized single-stranded template nucleic acid molecules on the array.

Applicant, therefore, asserts that resequencing within the limitations of the Parker et al.

reference does not achieve the objective of confirming "sequencing data of each of the immobilized single-stranded template nucleic acid molecules" as recited in step (e) of claim 4 because it is not possible to differentiate which sequence ladders derive from which double-stranded plasmid DNA molecules. Thus, the resequencing of Parker et al. fails to confirm sequencing data of a particular template within the population of templates sequenced, but rather compares sequencing data for populations of templates from different samples. In light of the above, the Office Action has failed to provide rationale to support the assertion of an alleged motivation to combine the teachings of Balasubramanian et al., Cheeseman, Soper et al., and Parker et al. to arrive at the claimed method. For rationale, the Examiner again perfunctorily states that the claimed method steps were known in the prior art and one skilled in the art could have arrived at the claimed invention by combining the steps that were taught in the prior art. As asserted herein above, such statements fail to meet the standard for showing "some articulated reasoning with some rationale underpinning to support the legal conclusion of obviousness." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007), at 1741. The Office Action has, therefore, failed to establish a *prima facie* case of obviousness.

In view of the above arguments, the Examiner is respectfully requested to reconsider the validity of the rejection of claims 4, 27-28, 30-31, and 33 under 35 U.S.C. §103 and withdraw the rejection.

Claim 29 is rejected under § 103(a) as allegedly unpatentable over Balasubramanisn [*sic*] (i.e., Balasubramanian et al.; WO 01/57248; published 9/2001) as evidenced by Cheeseman (USPN 5,302,509; issued 1994) in view of Soper et al. (USPN 5,846,727) and Parker et al. (USPN 5,565,323) as applied to claims 4 and 27 above and further in view of Lackey et al. (USPN 5,652,126). In view of the clarifying amendments to the claims and arguments presented herein, this rejection is respectfully traversed.

The Examiner recognizes that the combined teachings of Balasubramanian et al. (evidenced by Cheeseman), Soper et al., and Parker et al. do not teach a method wherein the double stranded anchor (which acts as a primer) comprises a recognition site for a restriction endonuclease. The Examiner relies on Lackey et al. for allegedly teaching a method that

comprises synthesizing a complementary copy nucleic acid sequence using a template sequence. In a particular embodiment wherein a DNA primer/template with a single 3' ribonucleotide is used, the Office Action indicates that cleavage at the ribonucleotide residue, followed by separation and purification of the oligonucleotide product, results in a fully regenerated and reusable primer/template.

As indicated herein above, the Office Action has failed to provide sufficient evidence to support the contention that an ordinarily skilled artisan would have had any motivation to combine the teachings of Balasubramanian et al., Cheeseman, Soper et al., and Parker et al. to arrive at the instantly claimed methods. Arguments directed to this point are set forth above and are incorporated herein in their entirety in connection with the asserted rejection of claim 29. It is, moreover, noteworthy that the deficiencies of Balasubramanian et al., Cheeseman, Soper et al., and Parker et al. are not remedied by the teachings of Lackey et al. The Office Action has, furthermore, failed to provide rationale to support the contention that it would allegedly have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Balasubramanian et al., Soper et al., and Parker et al. by using a double stranded anchor that acts as a primer and comprises a recognition site for a restriction endonuclease.

As stated by Applicant in previously presented arguments, Lackey et al. are silent with respect to sequencing the templates utilized therein to generate phosphorothioate oligonucleotides. The method of Lackey et al. is directed to cleaving phosphorothioate oligonucleotides to generate relatively cleavage resistant phosphorothioate oligonucleotides having properties that facilitate their separation and purification after synthesis. This reference has nothing to do with sequencing. There is, indeed, no reason to teach or suggest sequencing a template used for this purpose because the sequence of the template is already known. Why then, would one of ordinary skill in the art have been motivated to combine such disparate methods as those described in Lackey et al. to generate relatively cleavage resistant phosphorothioate oligonucleotides with those of Balasubramanian et al., Cheeseman, Soper et al., and Parker et al, which are unrelated to a method of generating such oligonucleotides, to allegedly arrive at the present invention? In the absence of such teaching, the Examiner has failed to establish a nexus

that substantiates the potential for an ordinarily skilled artisan to arrive at the alleged combination.

In view of the above arguments, the Examiner is respectfully requested to reconsider the validity of the rejection of claim 29 under 35 U.S.C. §103 and withdraw the rejection.

Claim 32 is rejected under 35 USC § 103(a) as allegedly unpatentable over Balasubramanisn [*sic*] (i.e., Balasubramanian et al.; WO 01/57248; published 9/2001) as evidenced by Cheeseman (USPN 5,302,509; issued 1994) in view of Soper et al. (USPN 5,846,727) and Parker et al. (USPN 5,565,323) as applied to claims 4 and 31 above and further in view of Barnes (WO 01/57249; published 8/2001). In view of the clarifying amendments to the claims and arguments presented herein, this rejection is respectfully traversed.

The Examiner acknowledges that the combined teachings of Balasubramanian et al. (evidenced by Cheeseman), Soper et al., and Parker et al. do not teach a method wherein the fluorescent nucleotides are detected using a microscope with total internal reflection based imaging. The Examiner relies on Barnes for teaching that using total internal reflection fluorescent microscopy makes it possible to achieve wide field imaging with single polymer sensitivity. The deficiencies of the combined teachings of Balasubramanian et al., Cheeseman, Soper et al., and Parker et al. are, however, set forth in detail above and incorporated herein by reference in their entireties and the teachings of Barnes fail to compensate for the aforementioned defects of these references in combination. The Office Action has thus failed to provide rationale to support the contention that it would allegedly have been obvious to one of ordinary skill in the art at the time of the invention to modify the combined methods of Balasubramanian et al., Cheeseman et al., Soper et al., and Parker et al. to use total internal reflection fluorescent microscopy, as taught by Barnes, to arrive at the present invention.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection of claim 32 under 35 U.S.C. §103.

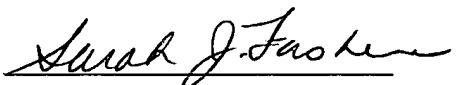
Fees

No additional fees are believed to be necessitated by this amendment. However, should this be an error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment or to credit any overpayment.

Conclusion

It is submitted, therefore, that the claims are in condition for allowance. No new matter has been introduced. Allowance of all claims at an early date is solicited. In the event that there are any questions concerning this amendment, or application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,



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